IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Declaration of Alemseged Truneh
Ruben Exhibit #59

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Paper No. _____

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application No. 08/816,981),

v.

STEVEN R. WILEY
and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

DECLARATION OF ALEMSEGED TRUNEH

Ruben EXHIBIT 2059
Ruben v. Wiley et al.
Interference No. 105,077
RX 2059

DECLARATION OF ALEMSEGED TRUNEH UNDER 37 C.F.R. § 1.608(b)

I, Alemseged Truneh, Ph.D., declare and state as follows:

1. I was employed by GlaxoSmithKline (formerly SmithKline Beecham or "SB") until September 30, 2001 and acted in a scientific and management role for SB during the time period discussed below. I am currently a scientist at Antigenics Inc. A copy of my curriculum vitae is attached as exhibit RE60 . I have been asked by patent counsel to Human Genome Sciences ("HGS") to describe the collaboration between HGS and SB and activities relating to TL2 (referred to as AIM-I at HGS) up through March 14, 1996.

2. A great deal of activity was ongoing from early 1995 until March 14, 1996 among the members of a large group participating in a joint venture of HGS and SB designed to identify and characterize members of the TNF ligand and TNF receptor families (the "HGS/SB Joint Program"). To better define the mechanism of ligand-receptor interactions of the TNF ligand and receptor families and understand their role in health and disease, including TL2 apoptotic activity, it was important to identify as many TNF ligand and receptor family members as possible.

3. The activities discussed herein are believed to be a significant underestimate of the actual number of days worked relating to TL2 which flowed from the activities of the HGS/SB Joint Program. Accordingly, the days worked at SB relating to TL2 should be considered a minimum number supportable by the documentary evidence referred to. It is certain that more days than are summarized herein were actually worked on the TNF/TNFR superfamily project in general and on TL2 in particular.

4. The October 18, 1995 HGS/SB meeting minutes and supporting materials provide a detailed overview of the tremendous amount of activity being jointly conducted at HGS and SB in order to elucidate and understand the structure and function of TL2 and related ligands, receptors, and receptor-associated proteins comprising the TNF/TNFR cell signal transduction network and is referred to extensively below (RE71). Work relating to TL2 was being conducted by scientists at HGS, and myself, Kong B. Tan, Edward R. Appelbaum, and Edward Dul, each of SB, and others at SB as well (RE76, page 1; RX 2071, pages 1-2).

5. A "TNF" database, which was maintained as an online spreadsheet by SB, provides the earliest written SB documentation available regarding activities of HGS/SB Joint Program members; RE72 is a table extracted from that database (the column headers in RE72 are actually truncated versions of the full header title; this truncation is an artifact of the printing method). As early as July 1, 1994, Peter R. Young of SB submitted a request for full sequencing of TL2 ("HTPAN08") (see third row of TNF spreadsheet) (RE72) to confirm its sequence. This request was approved on July 11, 1994 and completed on July 25, 1994. The Reviewer Name was "Christine Deb" (*i.e.* Christine Debouck) of SB, the "Date PI Assi" (*i.e.* Date PI Assigned) was July 12, 1994, and the date of "HGS Comme" (*i.e.* HGS Comments) was July 25, 1994. On page 3 of the TNF spreadsheet, Steven Ruben is listed as the HGS contact in connection with the TL2 sequence indicating that, very early in the project, SB scientists were directed to Ruben for guidance regarding TL2 (RE72). The results of the July 1, 1994 sequencing request by Young provide independent corroboration of the full-length TL2 sequence. The TNF spreadsheet also shows that, on February 28, 1995 and April 4, 1995, samples of clone HTPAN08 were requested by K. B. Tan and myself, and on June 9, 1995, a sample of clone HTPAN08 was requested by Yen Sen Ho of SB (RE72).

6. A number of internal SB e-mails further memorialize activities that were ongoing at SB in connection with TL2 prior to the October 18, 1995 meeting. On March 13, 1995, K. B. Tan of SB sent a copy of the TL2 sequence to Arun Patel of SB and copied me (RE73 . On July 18, 1995, Patricia M. Dormer of SB sent to me a request for information regarding proposed HGS third party collaborations for SB approval involving the TNF/TNFR-related molecules (RE74). On July 21, 1995, I sent to Mark R. Hurle of SB information regarding proposed HGS third party collaborations for SB approval involving the TNF/TNFR-related molecules (RE75).

7. On October 13, 1995, I prepared and circulated an agenda for the upcoming October 18, 1995 HGS/SB meeting. The agenda was distributed to five employees at HGS and 24 employees at SB (RE76 , page 1). The topics slated for discussion included updates on progress relating to (a) sequence analysis, (b) potential TNF receptors and ligands in the HGS database, (c) tissue and cell distribution of novel TNF/TNFR-related genes, (d) expression of soluble ligands, (e) construction and expression of Ig-fusion proteins of TNFR homologs and plans for making antibodies, (f) biological activity of novel TNF/TNFR molecules, and (g) the cloning of Fas and Fas ligand (RE76 , page 2). Joint meeting participants were reminded to bring copies of their overheads and a summary of their key findings with them for inclusion in the minutes (RE76 , page 2), which were prepared by Peter Young and myself. The working party distribution list for the October 18, 1995 meeting minutes included 33 individuals at SB and four individuals at HGS, emphasizing the scope and importance of this event and the underlying activities to both companies (RE76 , page 1). In actual attendance at the meeting were 17 individuals from SB and three individuals from HGS (RE71 , page 2).

8. At the October 18, 1995 meeting, which I co-chaired, Peter Young of SB presented SB's overall strategy, which Peter Young and I had developed, for identification of gene function for TNF/TNFR related genes (RE71 , page 5). This strategy, presented by Peter Young, was referred to as the "critical path" for analysis of the TNF/TNFR-related genes (RE71 page 5). Ligands and receptors were considered together every step of the way in the eight-step critical path strategy. First cDNA/EST sequences are identified in the HGS database from similarity to known TNF/TNFR family members. Second, RNA expression analysis is conducted using identified cDNA/ESTs as probes to determine what tissues and cells in normal and disease states express the genes corresponding to the cDNA/ESTs. Third, full-length cloning and sequencing of gene family members is conducted. Fourth, expression of full length proteins from recombinant sequences is performed. Fifth, antibodies are generated against ligand and receptor-Fc fusion proteins and screened, *e.g.*, for agonist activity. Sixth, ligands and receptors are localized and characterized in cells and tissues using a variety of techniques (*e.g.*, fluorescence activated cell sorting or FACS, immunoprecipitation, and Western blotting). Seventh, *in vitro* biology is investigated using isolated proteins and cells. Eighth, *in vivo* biology is investigated using cloned human genes and proteins in model rodent systems (rodent homologs are cloned if the human counterparts do not show function across species) (RE71, page 5). Using variations on this strategy, the group had, at the time of the October 18, 1995 meeting, identified and partially characterized eight novel receptors and three novel ligands in the TNF/TNFR family, including TL2 (RE71, page 2).

9. Shortly after the October 18, 1995 joint meeting, a new nomenclature for gene family members was adopted wherein novel homologs encoding receptors were designated TR's (for TNFR-related receptors) and those encoding ligands were designated TL's (for TNF-related

ligands) (RE71 , pages 3, 6, and 7). Under the new nomenclature, TL2 was referred to as TL2 (RE71 , page 7).

10. The eight novel receptors and three novel ligands that had been identified by the October 18, 1995 HGS/SB joint meeting included three splice variants of one receptor, TNFR-L2 (*i.e.*, TR2, TR2a, TR2b and TR2c) RE71 , pages 8-10). Progress was also reported by Sally Lyn concerning TR2 cloning and expression in a mammalian IgG1 Fc-fusion vector RE71 , pages 4, and 12-21). TNFR-related receptors (TRs) can be fused to Fc antibody domains using, *e.g.*, the COSFclink plasmid. Such expression constructs are useful for a variety of purposes, *e.g.*, as tools for expression of the novel receptors as soluble proteins linked to a readily-identifiable Fc domain. Using such constructs, a large number of cell types can be rapidly screened by flow cytometry to identify cells expressing surface ligands which bind to the soluble TR-Fc fusions. Such constructs are also useful for the immunization of mice for the production of monoclonal antibodies (mAbs). The latter use was made of a TR-Fc fusion of "TNFRL2" by SB scientist Steven Holmes and discussed at the October 18, 1995 meeting RE71 page 12). See also, Monthly Report/October 1995 (partially redacted) (RE77 .

11. A TR-Fc fusion protein is also useful as a soluble form of the receptor molecule which can be employed to identify and clone the gene encoding the ligand specific for the receptor. This approach to matching up ligand-receptor pairs in the TNF/TNFR superfamily was well known in the art at the time of the invention. Indeed, this approach was used to identify the ligand for the T cell receptor gene 4-1BB. *See* Goodwin *et al.*, 1993, Molecular cloning of a ligand for the inducible T cell gene 4-1BB: a member of an emerging family of cytokines with homology to tumor necrosis factor, Eur. J. Immunol. 23, 2631-2641 (RE78 .

12. The predicted membrane topology of TL2 was considered relative to the other two novel ligands that had been identified at the time of the October 18, 1995 joint meeting (RE71, page 11). This analysis was done by Mark Hurle at my request. We needed to know how these molecules compared to the known members of the family such as TNF α, and whether they were secreted proteins or membrane-anchored.

13. Also during the October 18, 1995 joint meeting, HGS scientist Jian Ni reviewed the known members of the TNF/TNFR superfamily, as well as recently-described associated proteins (RE71 pages 2, and 22-51). At that time, Dr. Ni reported that TL2 (referred to as "rat Fas ligand like molecule") had been expressed in *E. coli* and that a polyclonal antibody had been generated (RE71, page 22). Experiments in progress at that time included expression and purification of the TL2 protein in baculovirus, COS and CHO cells, and functional studies (RE71, page 22). The novel proteins that had been identified were considered as a group along side known proteins to assist in the evaluation and assignment of physiologic roles (RE71, pages 24-35). Indeed, a large number of novel protein alignments with known proteins were presented and discussed at the October 18, 1995 meeting (RE71, pages 36-50).

14. SB scientist Kong B. Tan and I reviewed for the group the tissue and cell distribution of five novel TNF/TNFR genes as determined by RNA blot analysis. (RE71 pages 52-58). Dr. Tan also reported results from TNFRL2-Ig fusion protein binding to various cells lines (RE71, page 59). The RNA blots showed that TL2 (*a.k.a.* AIM-I, TNFL1, 413412, HTPAN08, and/or ATG343) displayed strongest expression in KG1a myeloid cells, Jurkat T cells, $CD4^+$ and $CD19^+$ primary cells, and heart and bone marrow tissue (RE71, page 53). TL2 expression was also detected in TF274 and HOS (TE85) bone marrow stromal cells, HL60 and THP-1 myeloid hematopoietic cells, and human lung, spleen, kidney, prostate tissue and

$CD8^+$ cells RE71, pages 54-55). The actual RNA blots showing these results can be seen in the lower right, and upper right panels, respectively (RE71, pages 56-58).

15. Edward R. Appelbaum and Edward Dul of SB reported the expression of TL2 ("the rat Fas ligand homolog") in two epitope-tagged fusion constructs at the October 18, 1995 meeting (RE71 page 2). The objectives of these experiments were: (a) to express a fusion protein in *E. coli* to be used for raising antibodies; and (b) to express a soluble form of TL2 in *E. coli* or other systems to be used for receptor binding and activity assays RE71, pages 60-62). At the time of the October 18, 1995 meeting, the plasmid DNA encoding TL2 had been re-sequenced by SB and it had been determined that the open reading frame sequence agreed with that provided by HGS. Both epitope-tagged TL2 constructs prepared by SB expressed insoluble proteins well. Each of these constructs was designed to express a protein having an enterokinase digestion site, thus permitting release of an untagged protein following cleavage with enterokinase (RE71 pages 60-62).

16. Subsequent to the October 18, 1995 meeting, my colleagues and I prepared a monthly report for Gordon Moore of SB (RE77). This report repeated some of the information from the October 18, 1995 meeting and also indicated that the TNF/TNFR group agreed to conduct similar joint meetings every three months going forward (RE77). The report further summarized progress of the group through October 1995 and indicated that at least 12 new genes from the ATG/HGS database had been identified, including TL2 (referred to as "TL2", "413412," "HTPAN08" or "ATG343") (RE77).

17. Additional activities relating to TL2 followed the October 18, 1995 meeting. For example, copies of additional internal SB e-mail messages and documents establish that on October 20, 1995, K. B. Tan prepared a summary of RNA expression studies (RE77

pages 3-4). On October 24, 1995, Mark R. Hurle of SB sent a copy of the overheads describing "Novel TNF Receptors and Ligands," including TL2, to me (RE79). October 30, 1995, Hurle sent to Edward R. Gimmi of SB requested information on the novel genes, including TL2 ("413412" or "HTPAN08") (RE80)). On November 1, 1995, I sent an e-mail to Jian Ni of HGS requesting TNF/TNFR protein and antibody reagent samples for evaluation in binding and functional studies, including a request for antibody recognizing TL2 ("TL2" or "HTPAN08") (RE81). On November 14, 1995, Jian Ni of HGS shipped the requested TL2 antibodies to me (RE82). On February 13, 1996, I circulated an e-mail to Peter R. Young and John C. Lee, both of SB, concerning a draft agenda for a proposed February 19, 1996 TNF/TNFR team meeting (RE83). The draft agenda slated discussion of TL2 expression by Edward Appelbaum and Yen-Sen Ho, both of SB. On February 26, 1996, Peter R. Young of SB sent Reiner Gentz of HGS an agenda for a February 28, 1996 TNF/TNFR team meeting. The agenda indicated that SB speakers would be Young, Terry Porter, Yen-Sen Ho and K. B. Tan, and that other attendees would be Sally Lyn, Michele Gorczya and myself. Peter Young was slated to discuss, *inter alia*, the expression of TL2 ("TL2"). Kong B. Tan of SB was slated to discuss RNA blot analysis of TRs and TLs, including TL2. Yen-Sen Ho of SB was slated to discuss development of an apoptosis assay (RE84). On March 18, 1996, Terence G. Porter of SB circulated an e-mail to Edward R. Appelbaum, Peter H. Young, Edward Dul, Seth M. Fisher (all of SB) and myself, concerning the TL2 protein ("TL2") (RE85).

18. In view of the documentary evidence which I have reviewed above, and based on my personal involvement and responsibilities in the HGS/SB Joint Program, I believe that it is a fair statement to say that activities relating to TL2 were virtually continuous throughout the critical period from just prior to June 29, 1995 until March 14, 1996. It was known by June of

1995 that the TNF family of ligands was involved in a complex system of regulation of immune cell proliferation and activity effected through the binding of each ligand to one or more TNF receptor family members. It was also known at the time that TL2, cloned and isolated first at HGS by Steven Ruben, was a member of the TNF ligand family and had homology to Fas ligand. Accordingly, it was apparent to me that TL2 would have apoptotic activity. Thus, to better understand the role and mechanism of TL2 apoptotic activity, it was important to identify TNF receptor family members to ultimately determine which receptors did and did not bind TL2. Also key to such an analysis of TL2 function was to compare and contrast the expression patterns of the various members of both the TNF ligand and TNF receptor families, and thereby identify the likely target receptor and target cell line for TL2. Therefore, work carried out by HGS and SB scientist from June 1995 through March 1996 on various TNF ligand and receptor family members, such as ligands TNF gamma, delta, and epsilon, and receptors TR1, TR2, and TR3, all contributed to further elucidation of TL2 biological function.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing thereon.

Date June 24, 2004 _____
Alemseged Truneh